Effects of Anion-Transport Inhibitors on NaCl Reabsorption in the Rat Superficial Proximal Convoluted Tubule

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ABSTRACT The effects of anion-transport inhibitors on volume reabsorption, and total CO_2 concentrations were examined by in vivo microperfusion of superficial proximal convoluted tubules of rats. The luminal perfusion solution was a high-chloride, low-bicarbonate solution like that in the in vivo late proximal tubule. The anion-transport inhibitors were only added to the luminal perfusion solutions.

In tubules perfused with the control high-chloride solution, the rate of volume reabsorption (J_v) was 2.3 ± 0.2 nl/mm·min ($n = 18$), and the collected total CO_2 concentration was 4.0 ± 0.3 mM. Furosemide (3 mM) caused a marked reduction in volume reabsorption to 0.8 ± 0.3 nl/mm·min ($n = 20$) and only a slight increase in the total CO_2 concentration of collected samples of perfusate (7.8 ± 0.5 mM). 0.8 mM acetazolamide caused a more pronounced rise in the collected total CO_2 concentrations to 10.7 ± 0.5 mM but only a slight fall in J_v to 1.7 ± 0.3 nl/mm·min ($n = 19$). Hence, we inferred that inhibition of carbonic anhydrase only partially accounted for the inhibition of J_v by furosemide. 4-acetamido-4'-iso-thiocyanato-stilbene-2,2'-disulphonic acid (0.1 mM), a well-characterized inhibitor of erythrocyte anion exchange mechanisms, also reduced J_v to 1.6 ± 0.3 nl/mm·min ($n = 15$) without changing the total CO_2 concentrations of the collected perfusates (3.6 ± 0.4 mM). The effect of 4-acetamido-4'-iso-thiocyanato-stilbene-2,2'-disulphonic

acid on volume reabsorption could not be explained by carbonic anhydrase inhibition because there was no increase in the total CO_2 concentration of the collected fluids. Furosemide did not significantly inhibit the rate of tracer glucose efflux out of the tubules, which suggests that the effect of furosemide on volume reabsorption was not a result of some non-specific depression of active sodium transport. These results are discussed with respect to the possible effects of anion-transport inhibitors on the paracellular shunt pathway, active sodium reabsorption, and neutral sodium chloride transport.

INTRODUCTION

The mechanisms and pathways of sodium chloride reabsorption by the proximal tubule have not been clearly defined. There is general agreement with the importance of active sodium transport, and with the possibility that sodium chloride reabsorption in the late proximal tubule could result, in part, from passive driving forces. However, the relative contributions of active and passive reabsorptive processes is currently controversial.

Rector et al. (1-5) have proposed that active sodium reabsorption is that fraction which is directly coupled to H^+ secretion and nonelectrolyte reabsorption. According to this view, reabsorption of sodium chloride and water in the late proximal tubule is driven primarily by passive forces (i.e., the lumen to blood chloride gradient established by bicarbonate reabsorption). Fromter et al. (6), Schafer et al. (7), and Maude (8) have also considered the importance of passive reabsorption of salt and water in the proximal tubule. A common feature to all of these "passive" models has been the assumption, either explicit or implicit, that the passive reabsorption of salt and water proceeds through the paracellular shunt pathway of the proximal tubule.

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A more traditional view of the proximal tubule holds that the primary transport process is the active, electrogenic transport of sodium from lumen to blood (9, 10). The pathway of sodium transport is explicitly viewed as transcellular according to this school of thought. The attendant chloride moves passively, driven by the electrical force arising from the active sodium transport process, although not necessarily via a specific transcellular route from lumen to blood.

A third alternative is the neutral reabsorption of sodium chloride by the proximal tubule. This process fundamentally relies on active sodium transport, and has been considered for the gut, gall bladder, and proximal tubule (11–19). These models presume that the route of neutral sodium chloride reabsorption is through the cells of the transporting epithelium, and specify that chloride entry cannot be accounted for by the electrochemical potential gradient for chloride across the luminal membrane.

Recent studies of reabsorption in the proximal tubule by Ullrich et al. (20), Neumann and Rector (4), and Schafer et al. (21) demonstrated the importance of concentration gradients for chloride and bicarbonate between the lumen and peritubular capillaries (or bath). In contrast, advocates of neutral sodium chloride reabsorption (18) have not attributed particular importance in proximal reabsorption to the chloride and bicarbonate concentration gradients which normally exist along most of the length of the proximal tubule. Many studies have shown that neutral sodium chloride reabsorption can occur when the sodium chloride concentrations are the same on both sides of various epithelia (11, 12, 14–19). Turnberg et al. (11) have shown in the human ileum that such a process can even lower the luminal sodium chloride concentration below that of blood when an impermeant solute was included in the luminal solution. In marked contrast, the study by Neumann and Rector (4) showed in the rat superficial proximal convoluted tubule that volume reabsorption stopped in the absence of a favorable anion gradient between lumen and blood. Hence, if neutral sodium chloride reabsorption occurs in the rat superficial proximal convoluted tubule, then a specific model must be proposed which incorporates a role for the anion concentration gradients which exist along the length of the proximal tubule.

The purposes of our studies were to further investigate the mechanisms of sodium reabsorption, and to estimate the possible contribution of neutral sodium chloride transport to the transepithelial reabsorption of salt and water by the superficial proximal convoluted tubule. Absolute rates of volume reabsorption were determined in rat superficial proximal convoluted tubules which were perfused in vivo with a high-chloride, low-bicarbonate solution resembling late proximal fluid. The total CO_2 concentrations (i.e.,

dissolved CO_2 plus bicarbonate) of initial and collected luminal perfusion solutions were measured by microcalorimetry (Picapnotherm, [22]).

Two inhibitors of anion-transport processes, furosemide and 4-acetamido-4'-iso-thiocyanato-stilbene-2,2'-disulphonic acid (SITS,¹ BDH Chemicals, Ltd., Poole, England) were added to luminal perfusion solutions. Both decreased the rate of volume reabsorption by a mechanism which did not collapse the anion gradients between lumen and blood. In contrast, maximal concentrations of acetazolamide raised the luminal total CO_2 concentrations but only caused a slight decrease in the absolute volume reabsorptive rate per perfused length (J_v). Furosemide did not significantly inhibit glucose efflux out of the tubules. This result suggests that the inhibition of volume reabsorption by this drug could not be explained by a generalized inhibition of the metabolic state of the cells or by inhibition of electrogenic sodium transport. These results are consistent with an effect of furosemide and SITS on neutral sodium chloride transport, but do not exclude a possible effect of these agents on the conductance of the paracellular shunt pathway.

METHODS

Volume reabsorptive rates of superficial proximal convoluted tubules were measured during in vivo microperfusion experiments. The studies were performed on male Wistar rats weighing 162–227 g. The rats were anesthetized with an intraperitoneal injection of Inactin (Promonta, Hamburg, West Germany) (100 mg/kg). They were placed on a heated animal table where their body temperatures were maintained at 37°C. Femoral arterial blood pressure was monitored, and the rats were given an infusion of 0.9% NaCl at 1.2 ml/h through a jugular venous catheter. Arterial blood samples (270 μl) were taken for measurement of pH and PCO_2 , (Corning Blood Gas Analyzer model 165, Corning Glass Works, Science Products Div., Corning, N. Y.). A flank incision exposed the left kidney which was then immobilized in a Lucite (E.I. DuPont deNemours & Co., Inc., Wilmington, Del.) cup. The surface of the kidney and abdominal contents were bathed with water-equilibrated paraffin oil which was heated to 37°C and bubbled with a 5% CO_2 , 95% O_2 mixture. An intravenous injection of 0.02 ml of 10% Lissamine green dye was given to measure proximal tubule transit time. Only those kidneys whose proximal transit times were 9–11 s were used.

A thermally insulated Hampel microperfusion pump (Wolfgang Hampel, Berlin, West Germany) was used to perfuse superficial proximal convoluted tubules at a rate of 13.2 ± 0.2 nl/min. The perfusion pipette was placed in the lumen of an early proximal segment. A column of castor oil stained with Sudan Black was injected with a second pipette into the lumen of a more proximal surface convolution, and the oil separated the glomerular filtrate from the perfusion solution. The glomerular filtrate escaped from the tubule

¹Abbreviations used in this paper: J_d^* , the rate of tracer glucose efflux per perfused length; J_v , the absolute volume reabsorptive rate(s) per perfused length(s); SITS, 4-acetamido-4'-iso-thiocyanato-stilbene-2,2'-disulphonic acid.

through the hole which remained after the second pipette was removed. The perfusion solutions contained 0.1% FD&C green dye No. 3, which allowed for identification of all perfused convolutions of the tubule. Timed total collections of perfusate were made with a third pipette from a later convolution of the same proximal tubule while maintaining a droplet of paraffin oil stained with Sudan Black just distal to the collection site. The exact perfusion rate for each tubule was calculated from the quantity of collected fluid and inulin concentrations in timed collections. After the studies were completed, the tubules were filled with liquid latex. The kidney was removed and incubated in 6 N HCl at 37°C for 70 min. The tubules were then dissected and photographed for measurement of tubule length.

The collected samples were kept under water-equilibrated paraffin oil at all times and transferred to calibrated constant bore pipettes for measurement of total volume. An aliquot of 13 nl was removed for measurement of total CO₂ concentration, and the rest was transferred to a counting vial for measurement of [¹⁴C]inulin. Dual-channel counting was used when both volume reabsorption ([¹⁴C]inulin) and tracer glucose efflux ([³H]glucose) were measured simultaneously. Total CO₂ concentration (i.e., dissolved CO₂ plus bicarbonate) was measured with microcalorimetry as described by Vurek et al. (22).

The perfusion solution was designed to simulate the luminal fluid of the in vivo late proximal tubule. It contained no glucose or amino acids, and it had high-chloride and low-bicarbonate concentrations. It contained the following, in millimolars: sodium, 154; potassium, 5; calcium, 1.8; magnesium, 1; chloride, 150; bicarbonate, 5; phosphate, 4; sulfate, 1; and urea, 5. When acetazolamide or furosemide was added to the perfusion solutions, the drug was first dissolved in a NaOH solution. The solution was then neutralized with HCl before the remaining components of the solution were added. The final pH of the solution was 7.1. 5 mM glucose replaced 2.5 mM sodium chloride in the perfusion solutions used for the study of tracer glucose efflux. These solutions also contained 100 µCi/ml of [³H]glucose (New England Nuclear, Boston, Mass., Lot 927-253). Solutions were bubbled at room temperature with a 5% CO₂, 95% O₂ gas mixture before use.

Calculations. The perfusion rate (V_0) for each tubule was calculated using:

$$V_0 = (C/P)(V_L), \quad (1)$$

where C was the inulin concentration in the collected fluid, P was the inulin concentration in the initial perfusion fluid, and V_L was the rate of collection of the perfusion fluid. Absolute volume reabsorption is the difference between V_0 and V_L . J_v was determined by plotting the absolute volume reabsorption vs. length of each tubule. J_v is equal to the slope of the line as determined by linear regression analysis. The 95% confidence limits of the slopes were also determined with linear regression analysis. The comparison of two regression lines was made by the analysis of covariance (23). The rate of tracer efflux per perfused length (J_g^*) was calculated for each tubule using:

$$J_g^* = (V_0 C_0^* - V_L C_L^*) / (C_0 / C_0^*) / L, \quad (2)$$

where V_0 and V_L were the perfusion and collection rates, C_0^* and C_L^* were the activity of tracer glucose in the perfused and collected samples, and C_0 was the cold glucose concentration in the initial perfusion solution. Eq. 2 assumes that the specific activity of tracer glucose remained constant along the length of the perfused tubule.

Data are presented as mean $\pm$ 1 SEM. Significant differences at the $P \leq 0.05$ level are indicated as such in the tables.

RESULTS

The rats used in these experiments had normal blood gas values. The mean arterial pH was 7.40 ± 0.02 , the PCO₂ was 37.9 ± 0.6 mm Hg, and the bicarbonate concentration was 22.6 ± 0.7 mM ($n = 15$). The calculated total CO₂ concentration of the systemic arterial plasma was 23.8 mM.

The results obtained with the high-chloride, low-bicarbonate perfusate are seen in Fig. 1. In the top panel, absolute volume reabsorption is plotted vs. tubule length. Tubule length ranged from 0.8 to 2.8 mm with a mean of 1.5 mm. The slope of the line corresponded to a J_v of 2.3 ± 0.2 nl/mm·min, similar to that obtained in previous studies of the in vivo, microperfused rat proximal convoluted tubule (4, 24). The lower panel of Fig. 1 shows the total CO₂ concentration of the collected samples vs. tubule length. The mean total CO₂ concentration in the initial perfusion fluid was 5.1 ± 0.1 mM (represented by the dashed horizontal line). The total CO₂ concentration was 4.0 ± 0.3 mM in collected samples, similar to that of the initial perfusate.

When 3 mM furosemide, an inhibitor of chloride transport and anion exchange mechanisms, was present in the luminal perfusate, J_v was decreased by 65% to 0.8 ± 0.3 nl/mm·min ($P \leq 0.001$, compared with control slope by analysis of covariance). These results are

FIGURE 1 Volume reabsorption and total CO₂ concentrations during control proximal tubule perfusion with high-chloride, low-bicarbonate solution, plotted against tubule length. The slope of the regression line in the upper panel represents J_v . Control J_v was 2.3 ± 0.1 nl/mm·min for 18 tubules. The dashed horizontal line in the lower panel represents the initial total CO₂ concentration (5.1 ± 0.1 mM) of the perfusates. The regression equation was: $y = -0.76 \pm 0.26 + (2.30 \pm 0.16)x$; $r = 0.963$.

FIGURE 2 Effect of luminal furosemide (3 mM) on volume reabsorption and total CO₂ concentrations in proximal tubules perfused with high-chloride, low-bicarbonate solutions. The shaded area in the upper panel represents the 95% confidence limit for the slope of the control rate of volume reabsorption. The open symbols (○) represent the control perfusions, and the closed symbols (●) represent perfusions with furosemide-containing solutions. Fig. 2 is otherwise similar to Fig. 1. The slope of the regression line for the furosemide-containing perfusates was 0.8 ± 0.3 nl/mm·min ($n = 20$), and the collected total CO₂ concentrations averaged 7.8 ± 0.5 mM ($n = 17$). The regression equation was: $y = 0.15 \pm 0.54 + (0.79 \pm 0.30)x$; $r = 0.520$.

illustrated in the top panel of Fig. 2, where the rate of volume reabsorption is compared with the 95% confidence limits of the rate of volume reabsorption in control perfusions (shaded area). The bottom panel of Fig. 2 shows that when tubules were perfused with a solution containing furosemide, the collected total CO₂ concentration (closed circles) rose slightly to 7.8 ± 0.5 mM. For comparison, the collected total CO₂ concentrations in control perfusion solutions are plotted with open circles.

It is possible that furosemide reduced J_v by inhibiting carbonic anhydrase activity. To test for this possibility, a high concentration of the carbonic anhydrase inhibitor, acetazolamide, was used. The effects of 0.8 mM acetazolamide in the luminal perfusate are illustrated in Fig. 3. J_v was reduced from the control value of 2.3 to 1.7 ± 0.3 nl/mm·min ($P \leq 0.001$, compared with control slope by analysis of covariance). Acetazolamide caused a more marked increase in collected total CO₂ concentration than furosemide, but in contrast, only a small decrease in J_v (0.8 ± 0.3 vs. 1.7 ± 0.3 nl/mm·min; $P \leq 0.001$ for the difference

between the furosemide and acetazolamide slopes, by analysis of covariance), as summarized in the first three rows of Table I. Hence, the reduction in J_v observed when furosemide was present in the perfusion solution cannot be totally explained by the carbonic anhydrase-inhibitory activity of the drug, or by collapse of the anion gradients between lumen and blood.

The mechanisms of salt and water reabsorption from a high-chloride, low-bicarbonate solution was further investigated by adding SITS to the luminal perfusion solution. The results of experiments in which tubules were perfused with a solution containing 0.1 mM SITS are illustrated in Fig. 4. J_v was significantly reduced from the control value of 2.3 to 1.6 ± 0.3 nl/mm·min ($P \leq 0.05$, compared with control slope by analysis of covariance). In contrast to both furosemide and acetazolamide, the total CO₂ concentration of collected samples (3.6 ± 0.4 mM) was not significantly higher than that of the initial perfusion solution (4.0 ± 0.3 mM) when 0.1 mM SITS was present in the perfusate. Thus J_v was decreased by SITS even though the anion gradient was completely unchanged along the length of the

FIGURE 3 Effect of luminal acetazolamide (0.8 mM) on volume reabsorption and total CO₂ concentrations in proximal tubules perfused with high-chloride, low-bicarbonate solutions. The closed symbols (●) represent perfusions with acetazolamide-containing solutions. Fig. 3 is otherwise similar to Fig. 2. The slope of the regression line for acetazolamide-containing perfusates was 1.7 ± 0.3 nl/mm·min ($n = 19$), and the collected total CO₂ concentrations averaged 10.7 ± 0.5 mM ($n = 18$). The regression equation was: $y = -0.42 \pm 0.48 + (1.74 \pm 0.33)x$; $r = 0.791$.

TABLE I
Reabsorptive Rates and Collected Total CO₂ Concentrations from Rat Superficial Proximal
Convolved Tubules Perfused at 13 nl/min

Solution	Number of tubules	Mean perfused length (±SEM)	Reabsorptive rate (±SEM)	Percent inhibition*	Total CO ₂ concentration (±SEM)	
					Perfused	Collected
		mm	nl/mm·min		mM	mM
High chloride						
Control	18	1.5±0.2	2.3±0.2	—	5.1±0.1	4.0±0.3 (13)§
Furosemide, 3 mM	20	1.7±0.1	0.8±0.3	65.2	5.2±0.6	7.8±0.5 (17) [¶]
Acetazolamide, 0.8 mM	19	1.4±0.1	1.7±0.3	26.1	5.5±0.3	10.7±0.5 (18) [¶]
SITS, 0.1 mM	15	1.9±0.2	1.6±0.3	30.4	4.0±0.3	3.6±0.4 (11)
Complete†						
Control	13	1.7±0.3	2.3±0.4	—	23.4±0.3	9.4±1.2 (8) [¶]
Acetazolamide, 0.8 mM	20	1.4±0.2	0.8±0.2	65.2	22.7±0.3	25.3±0.6 (17) [¶]

* Percent inhibition of J_v was calculated as 100 × (Control-Experimental)/Control.

† Ultrafiltrate-like artificial perfusion solution, data from (37).

§ The number in parentheses refers to the number of tubules for which both J_v and total CO₂ measurements were made.

^{||} Significantly different from the control rate of volume reabsorption by analysis of covariance (24).

[¶] Significantly different from the total CO₂ concentration of the initial perfusate by paired *t* test.

tubules. These data are summarized in the fifth row of Table I.

The effects of furosemide upon electrogenic sodium reabsorption were examined in the last series of

FIGURE 4 Effect of luminal SITS (0.1 mM) on volume reabsorption and total CO₂ concentrations in proximal tubules perfused with high-chloride, low-bicarbonate solutions. The closed symbols (●) represent perfusions with SITS-containing solutions. Fig. 4 is otherwise similar to Fig. 2. The slope of the regression line for the SITS-containing perfusates was 1.6±0.3 nl/mm·min (*n* = 15), and the collected total CO₂ concentrations averaged 3.6±0.4 mM (*n* = 11). The regression equation was: $y = -0.76 \pm 0.65 + (1.58 \pm 0.33)x$; *r* = 0.802.

tubules by determining the effect of this agent upon J_v^{*}. The tubules were perfused with high-chloride solutions in which 5 mM glucose replaced 2.5 mM NaCl. These results are presented in Table II. The perfused lengths and initial perfusion rates were the same for the control series of 6 tubules and the 10 which were studied with a furosemide-containing perfusion solution. There was no increase in the collected/perfused tracer glucose concentration when furosemide was present in the perfusate (0.30±0.06 [*n* = 6] vs. 0.30±0.09 [*n* = 10]). There was a slight (22.2%), but statistically insignificant decrease in J_v^{*} compared with the control series of tubules. There was also a 64.3% fall in J_v with the furosemide series (0.5±0.2 nl/mm·min [*n* = 10]) compared with the control series (1.4±0.6 nl/mm·min [*n* = 6]). These J_v were calculated from the individual values for each tubule, as not enough tubules were studied to permit analysis by linear regression analysis. The mean J_v values and the percent reduction in J_v obtained with furosemide were very similar to the values obtained in the high-chloride series with and without furosemide.²

² The average of the individual values of J_v for the control high-chloride study (Table I, row 1) was 1.7±0.1 nl/mm·min. Accurate determination of J_v by linear regression analysis requires data from an adequate number of tubules. The difference between the mean of the individual values (1.7) vs. the regression slope (2.3 nl/mm·min) is a result of the fact that the regression had a significantly negative intercept (−0.8±0.3 nl/min). This result has been observed in other in vivo microperfusion studies of the rat proximal convoluted tubule (4, 24). The mean of the individual values for the high-chloride furosemide series was 0.9±0.1 nl/mm·min, which is comparable to the slope of the regression analysis of this series of 0.8±0.3 nl/mm·min (Table I, row 2).

TABLE II
Reabsorptive Rates and Collected:Perfused Concentration Ratios of [^3H]Glucose from
Rat Superficial Proximal Convolute Tubules

Condition	Number of tubules	Mean perfused length*	Mean perfusion rate*	Collected:perfused concentration ratio*	J _g * Mean tracer glucose efflux*†	Percent inhibition§
		mm	nl/min		pmol/mm · min	%
Control	6	1.5±0.2	14.5±0.4	0.30±0.06	34.7±4.0	
Furosemide¶	10	1.7±0.2**	13.4±0.6**	0.30±0.09**	27.0±2.3**	22.2**

* Mean±SEM.

† J_g* calculated according to Eq. 2.

§ Percent inhibition of J_g* was calculated as 100 × (control – furosemide)/control.

^{||} High-chloride perfusion solution; 5 mM glucose replaced 2.5 mM NaCl.

¶ High-chloride perfusion solution with 3 mM furosemide; 5 mM glucose replaced 2.5 mM NaCl.

** Not significantly different from control at the 0.05 confidence level with unpaired *t* test.

DISCUSSION

We have used furosemide, SITS, and acetazolamide to examine the mechanisms of sodium chloride reabsorption in the rat superficial proximal tubule. Furosemide inhibits chloride transport in several systems. In studies of the isolated, perfused, rabbit thick ascending limb of Henle, electrogenic chloride transport was inhibited by low doses (1–10 μM) of furosemide in the lumen (25). Radtke et al. (26) examined the effects of 3 mM furosemide on the rat proximal convoluted tubule and found that it significantly reduced J_v. Humphreys (27) found that 1 mM furosemide reduced volume reabsorption by 50% in the perfused rat ileum. Similar doses of furosemide (1–5 mM) also inhibit chloride transport across the erythrocyte membrane (28, 29), primarily by inhibiting an anion exchange and transport in many physiological systems (30–36). Acetazolamide, on the other hand, is a potent inhibitor of carbonic anhydrase (37), but has no direct effect upon erythrocyte anion exchange mechanisms (28).

3 mM furosemide reduced J_v by 65% when present in a high-chloride luminal perfusate (Table I, row 2). SITS, another potent anion exchange-inhibitor (28, 30), also reduced J_v when proximal tubules were perfused with a high-chloride solution (Table I, row 4). The reduction in J_v was less than that seen with furosemide, consistent with the greater inhibitory effects of furosemide compared with SITS on anion exchange mechanisms (28–30, 34). Acetazolamide, which has no direct effect on erythrocyte anion exchanges (28), had only a slight effect on J_v in our studies (Table I, row 3). We suggest, on the basis of these results, that anion exchange mechanisms may play a role in reabsorption of a high-chloride solution in the rat superficial proximal convoluted tubule.

However, we must consider the possible effects of these agents upon passive modes of sodium chloride

reabsorption, including effects upon the permeability of the paracellular shunt pathway and effects upon the anion gradients between lumen and blood. These agents may also affect electrogenic as well as neutral modes of active sodium transport.

Passive modes of NaCl reabsorption

The fluid in the late proximal tubule has a high-chloride and a low-bicarbonate concentration, which creates an anion gradient between the lumen and the peritubular capillaries. Neumann and Rector (4) showed that the high-chloride concentration in the lumen was required for volume reabsorption in the superficial proximal convoluted tubule of the rat. Their data supported the hypothesis (1–3, 5–8) that salt and water reabsorption in the late proximal tubule may occur passively, driven by the anion concentration gradients between lumen and blood. They suggested that whereas one-third of late proximal reabsorption occurred actively, the remaining two-thirds was a passive component driven by diffusion and convection, presumably through a paracellular pathway (4).

Permeability of the paracellular shunt pathway. Isotopic chloride permeability has been found to be moderately high in both the rat and rabbit proximal tubule (6, 7, 10). In view of these findings, numerous authors (4–7) have computed that passive driving forces (i.e., the anion concentration gradients, the electrical potential difference, and coupling to water flow) would be sufficient to account for chloride transport without involving any direct coupling to an active transport process. If furosemide or SITS increased the resistance of the paracellular shunt pathway, then the result predicted by passive models would be a fall in J_v.

Glucose-coupled electrogenic sodium transport generates a lumen-negative potential difference that favors the diffusion of chloride out of the tubule lumen (9, 38). Fromter and Gessner (38) found that 3 mM

furosemide did not inhibit the generation of a transport electrical potential difference by glucose in the proximal tubule of the rat. We also did not find a significant effect of the drug upon glucose transport in the rat proximal tubule (Table II). If furosemide does not change the rate of glucose-coupled electrogenic sodium transport and does not change the electrical potential difference due to transport, then this line of reasoning suggests, but does not prove, that furosemide does not decrease the chloride conductance of the paracellular pathway.

Anion gradients. Bicarbonate reabsorption from the ultrafiltrate in the early proximal tubule is catalyzed by carbonic anhydrase and generates a high chloride concentration in the lumen of the rest of the tubule. If carbonic anhydrase is inhibited, and bicarbonate reabsorption stops, then the generation of a high chloride concentration is prevented. According to the passive models (1–8) a large fall in J_v would be expected in the absence of the anion gradients. We have recently reported a 65% reduction in J_v in the rat proximal convoluted tubule when 0.8 mM acetazolamide was added to a “complete” perfusion solution which simulated an ultrafiltrate of plasma (37). These results are summarized in the last two rows of Table I, and indicate that carbonic anhydrase inhibition by acetazolamide can reduce J_v and prevent the generation of the anion gradients between lumen and blood.

The maintenance of the anion gradients along the late proximal tubule may require continuing reabsorption of any bicarbonate with leaks back into the lumen. Thus the passive models (1–8) would again predict that inhibition of carbonic anhydrase could cause a rise in the luminal bicarbonate concentration and a fall in J_v as a result of failure to maintain the anion gradients.

We evaluated the effects of carbonic anhydrase inhibition on J_v from a high-chloride solution by adding 0.8 mM acetazolamide to the luminal perfusate. The total CO_2 concentration rose to 10.7 mM from an initial value of 5.0 mM, and there was a 26% fall in J_v (Table I, row 3). In contrast, furosemide inhibited J_v by 65.2%, but only raised the collected total CO_2 concentration to 7.8 mM (Table I, row 2).³ SITS reduced J_v by 30%

³ Because 0.8 mM acetazolamide increased the concentration of total CO_2 in the collected samples of perfusate to 10.7 mM, the bicarbonate concentration gradient between lumen and blood was decreased by 6.7 mM, as compared with control. Similarly, the decrease in the bicarbonate concentration gradient caused by 3 mM furosemide can be estimated to be 3.8 mM. Therefore, if the fall in J_v is directly proportional to a decrease in the anion gradient, then the fraction of the reduction in volume reabsorption as a result of carbonic anhydrase-inhibition by 3 mM furosemide (39) compared with 0.8 mM acetazolamide can be estimated as $3.8/6.7 = 0.57$. Because 0.8 mM acetazolamide reduced J_v from the high-chloride solution by 0.6 nl/mm·min, the carbonic anhydrase-

but did not affect the anion gradient. Thus, the inhibition of J_v from a high-chloride perfusate seen with SITS and furosemide cannot be completely explained by inhibition of carbonic anhydrase with a collapse of the anion gradients between lumen and blood.

Radtke et al. (26) also found an inhibition of J_v in the rat proximal tubule when 3 mM furosemide or 1 mM acetazolamide was added to a luminal perfusion solution containing 15 mM sodium bicarbonate. Because the initial bicarbonate concentration was already 15 mM, the fall in J_v could have been a result of a further collapse in the anion gradients, or some other direct effect on sodium chloride reabsorption. Unfortunately, they did not measure the anion composition of collected samples of perfusate, so their results cannot be fully interpreted.

Effect of furosemide on active sodium transport

These considerations show that furosemide and SITS significantly inhibited J_v but did not dissipate the anion gradients which favored reabsorption. Alternatively, 3 mM furosemide may have inhibited active sodium transport, and this mechanism would then account for the majority of sodium reabsorption in the rat superficial proximal tubule. This thesis is quite different from passive models (1–6) which have attributed two-thirds of the reabsorption of sodium to a passive component in the rat superficial proximal convoluted tubule. Our estimate of the possible contribution of active sodium transport to J_v from a high-chloride perfusate is similar to that described by Schafer et al. (7) and Green (40).

If furosemide inhibited active sodium transport by a nonspecific effect upon the metabolic state of the cells (41–44), all modes of active sodium reabsorption would probably have been affected and our data could not be referred to any specific transport mechanism. In the last series of studies (Table II), we did not detect a statistically significant effect of furosemide upon tracer glucose efflux, which suggests that furosemide did not inhibit metabolic events in the tubule because the active glucose transport process was well maintained. Furthermore, because this electrogenic mode of sodium transport was not inhibited, the observed effect on J_v could reflect inhibition of nonelectrogenic (i.e., neutral) sodium transport. Others have also found a reduction in proximal reabsorption with furosemide (26, 45–51)

inhibitory activity of furosemide might have been expected to reduce J_v by 0.34 nl/mm·min. However, the total effect of 3 mM furosemide was to reduce J_v by 1.5 nl/mm·min. Even allowing for an effect on carbonic anhydrase activity, 3 mM furosemide still caused at least a 50% reduction in J_v from a high-chloride solution in the rat superficial proximal convoluted tubule.

but have not proposed an effect on neutral sodium chloride transport.

Neutral sodium chloride transport

Evidence from other "leaky" epithelia (e.g., small intestine, gall bladder, proximal tubule of *Necturus*) is consistent with neutral transepithelial transport of sodium chloride (11–19). The electrochemical potential gradient for sodium provides a driving force for chloride transport across the luminal membrane. Generally, these systems can accomplish transepithelial transport of sodium chloride at relatively rapid rates (52), and may even lower the luminal sodium chloride concentration below that of blood (11). In contrast, reabsorption ceases in the rat superficial proximal convoluted tubule if the luminal bicarbonate is replaced with a poorly reabsorbed anion (4). If neutral sodium chloride transport occurs in this system, then an important role must be considered for the anion gradients because reabsorption stops in their absence (4).

Another mode of neutral sodium chloride transport was suggested by Liedtke and Hopfer (13) from studies of brush border vesicles from rat small intestine. They proposed that parallel exchangers for sodium/proton and chloride/hydroxyl are located in the luminal membrane and that the operation of both results in the neutral transport of sodium chloride across that barrier. The model proposed by Liedtke and Hopfer (13) is similar to that considered by Turnberg et al. (11) for the human ileum, and is presented in Fig. 5. This model uses sodium/proton exchange to reabsorb sodium across the luminal membrane. The parallel chloride/hydroxyl exchanger provides a continuing delivery of base equivalents into the lumen. If chloride/hydroxyl exchange decreases in the absence of an elevated luminal chloride concentration, a limiting pH gradient could develop and inhibit further sodium/proton exchange. The driving force for the neutral entry of sodium chloride is provided by the electrochemical gradient for sodium across the luminal membrane. This model depends upon active sodium transport insofar as the sodium gradient across the luminal membrane is maintained by the $\text{Na}^+\text{-K}^+\text{-ATPase}$ activity of the peritubular membrane.

Neutral, coupled entry of sodium chloride occurs if the ionic exchange processes are both electrically neutral (13, 53). This model expands the role of sodium/proton exchange from titration of bicarbonate to a mechanism for movement of sodium across the luminal membrane along the entire superficial proximal tubule. Once bicarbonate is reabsorbed and the luminal chloride concentration has risen, sodium could still be reabsorbed in exchange for cellular protons. However, the net rate of hydrogen secretion would become zero because of buffering by the cellular base equivalents

FIGURE 5 Neutral sodium chloride reabsorption. Schematic model for neutral sodium chloride transport in the late proximal convoluted tubule. There is a parallel arrangement of Na^+/H^+ and Cl^-/OH^- exchangers in the luminal membrane. Sodium is transported across the peritubular membrane by $\text{Na}^+\text{-K}^+\text{-ATPase}$. Anion transport across the peritubular membrane involves both a conductive pathway for bicarbonate and another for $\text{Cl}^-/\text{HCO}_3^-$ exchange. CA, carbonic anhydrase activity.

that appear in the lumen in exchange for chloride. This point has been previously raised (54, 55) in consideration of the "coupling ratio" between hydrogen secretion and sodium reabsorption (36, 55).

The peritubular membrane may be an important site of action of inhibitors of anion exchange and transport. Fromter and Sato (56) proposed that there is a conductive pathway for bicarbonate, but not chloride, across the peritubular membrane of the rat proximal convoluted tubule. Similar conclusions have been suggested by a recent study of in vitro, isolated, perfused, rabbit proximal convoluted tubules (57). The turtle bladder also has a conductive pathway for bicarbonate diffusion across the serosal membrane (32, 33), and SITS inhibits anion transport on that side of either tissue (32, 33, 36). We cannot rule out an effect on the peritubular membrane in our studies because large concentrations of the drugs were used, and some fraction may have been reabsorbed and affected peritubular anion transport. Furthermore, Ullrich et al. (36) found that 1.0 mM SITS reduced J_v only when placed in both the lumen and peritubular capillary perfusion solutions, and did not detect an effect when the drug was only added to the luminal side. We cannot explain the differences between our results with SITS, and those reported by Ullrich et al. (36). However, a preliminary report from Green and Greenwood (58) supports our finding that SITS inhibits J_v when present in a high-chloride luminal perfusion solution.

We have included an anion exchanger in the peritubular membrane to obtain neutral chloride movement across that barrier. Other authors have also considered the possibility of anion exchange mechanisms in the peritubular membrane (59, 60). Duffy et al. (61)

have emphasized the symmetry of ionic transport processes across luminal and peritubular membranes. If neutral sodium chloride transport occurs across the luminal membrane, then a neutral exit step must also exist across the peritubular membrane. The model in Fig. 5 maintains charge balance across both membranes, and meets the requirements defined by Duffy et al. (61) for neutral sodium chloride transport.

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